REMARKS

Amendments

Applicants respectfully request entry of the above amendments as they place the claims in better condition for appeal and/or comply with requirements of form. Claim 1 is amended and claim 10 is canceled without prejudice or disclaimer to presenting the removed subject matter in a subsequent application. The amendments to claim 1 are supported throughout the application as filed and particularly by original claim 10 (50-fold concentration increase) and by the specification at examples 5 and 8 (adjusting the conductivity such that precipitation of the solution components induced by the organic polymer is substantially prevented or substantially reversed without removal of the organic polymer). No new matter has been added.

Entry of the amendments will not require new search or additional examination. Claim 1 is amended to include a dependent claim (claim 10) into claim 1, and claim 10 was already considered and fully examined. Thus, this amendment does not require new search or additional examination. The additional amendments to claim 1 specifying that the organic polymer is not removed only serve to clarify in the claim what was abundantly clear in the specification concerning the nature of the invention and therefore was the likely construction of claim step (2) as previously presented. Thus, this clarifying amendment does not raise new issues requiring additional searching or examination. Therefore, entry of the amendments after final rejection is believed to be appropriate.

Interview

Applicants thank the Examiner for the courtesy of a telephonic interview on November 3, 2009 to discuss the claims and the obviousness rejection. We discussed the high concentration obtained using the claimed process and the lower concentration shown in prior art ultrafiltration of solutions containing an organic polymer. The Examiner prepared the detailed Interview Summary.

Summary of the Invention

Use of ultrafiltration to concentrate macromolecules in solution with organic polymers has been limited by precipitation of solution components at the membrane. For example, excessive precipitation has prevented concentration by ultrafiltration of more than 10-20 fold over the protein concentration in the initial cell culture supernatant. Specification, page 1, lines 19-20. This problem was observed in solutions containing organic polymers such as Pluronic® block copolymers. Page 3, lines 9-14.

Applicants studied the nature and cause of the precipitation. They discovered a solution that allows for much greater macromolecule concentration, such as at least 50 or 100-fold higher concentration as compared to the starting solution. Applicants discovered that by first ultrafiltering the solution, then adjusting the conductivity, then further ultrafiltering, a much more concentrated solution can be obtained than they noticed in prior ultrafiltration techniques.

Obviousness Rejection

Claims 1-12 stand rejected as obvious over Moller et al. (US 6,103,502) in view of Schulz et al. (1997) and Palomares et al. (2000) and evidenced by Nemeth et al. (1997). The Office relies on Moller et al. as teaching a process of protein purification using a first ultrafiltration process that is immediately followed by diafiltration (desalinization) that reduces the conductivity to less than 2.2 mS/cm. The Office then states that Moller et al. discloses allowing that a second ultrafiltration process may be performed. Final Office Action mailed June 10, 2009 ("Office Action"), p. 3. The Patent Office admits that Moller et al. does not teach an organic polymer such as a Pluronic® block copolymer in the starting solution, or animal or insect cell cultures.

The Office relies upon Schulz et al. as teaching that ultrafiltration is an efficient process as a first step in downstream procedures for recovering proteins from mammalian cell cultivation, and that organic polymers such as Pluronic® F-68 are a common supplement in cell culture medium for animal cells. Office Action, p. 3. The Office further relies upon Palomares

et al. as teaching use of Pluronic® F-68 in insect cell culture. Nemeth et al. is cited as evidence of the generic name for Pluronic® F-68.

The Rejection Fails Because the Cited Art Does Not Suggest that Ultrafiltration Can Provide High Concentration in a Solution Having an Organic Polymer.

Applicants respectfully request withdrawal of the obviousness rejection because the cited art fails to teach ultrafiltration of solutions containing organic polymers resulting in the high concentration recited in the present claims.

Former claim 10 has been entered into claim 1. As amended, now all the claims recite that concentration is increased at least 50 fold (claims 1-9, 12 and 13) or at least 100 fold (claim 11). In contrast, Moller et al. disclose that after the diafiltration, "the product can be concentrated again, in order to arrive at a ***concentration of 8:1 in total***...." Col. 5, lines 31-33 (emphasis added). Eight to one is considerably lower than the concentration increase of at least 50 fold or at least 100 fold recited in the present claims. In fact, 8:1 falls within Applicants' discussion in the specification of the prior art as providing only a 10-20 fold concentration. *See* page 1, lines 19-20. Thus, Moller et al. does not teach or suggest the high concentration provided by the presently claimed process.

The Patent Office states that "it is the result of the extra ultrafiltration step that can be utilized which results in a higher concentration of molecules (col. 5, line 7-48)." Office Action, p. 12, discussing claims 10 and 11. Yet the cited example from Moller et al. allows for a concentration of 8:1 in total, not at least 50 fold or at least 100 fold as recited in the present claims. Moller et al. does not teach that an extra ultrafiltration step can increase concentration to at least 50 fold over the starting material, only that it can increase concentration from 6:1, which was obtained in the first ultrafiltration in the example in Moller et al., to 8:1, which was obtained after the second ultrafiltration. Thus, by going from 6:1 to 8:1, Moller et al. actually teaches that a second ultrafiltration step can provide only modest improvement after a first ultrafiltration. Accordingly, Moller et al. does not teach or suggest the high concentration obtained by Applicants' process. Furthermore, the modest improvement with a second ultrafiltration step

reported in Moller et al. does not provide an expectation of successfully achieving at least 50 fold or at least 100 fold increase by adding a further ultrafiltration step.

Additionally, because Moller et al. does not have an organic polymer in the macromolecule solution, it does not show the obviousness of high concentration from such a starting solution that was known to have precipitation problems. In contrast, the present claims require an organic polymer in the starting solution and that it not be removed in the step of adjusting conductivity prior to the second ultrafiltration.

This ability to highly concentrate a macromolecule solution containing an organic polymer such as Pluronic® block copolymers distinguishes the invention over the prior conventional processes. Applicants have shown in example 7 and Figure 16 that by starting with a macromolecule solution containing an organic polymer and following the claimed process, a concentration factor of 100 fold can be obtained with 100% protein yield. This is a significant increase over the 25-fold concentration increase and 90% protein yield observed with a conventional ultrafiltration process.

The Patent Office has previously examined and found obvious claims incorporating high final concentration (dependent claims 10 and 11). The Patent Office asserted that the recited high concentrations were "well within the noted standards and capabilities of the ultrafiltration method in and of itself and these concentration levels are well understood in the art (see for examples [sic], US 4900673, Ex. 4, US 5108920, Fig. 7 and US 51567602, Ex. 7.1)." Office Action, p. 12. Applicants respectfully disagree. As discussed in detail below, none of the three patents cited at the sections the Patent Office referred to disclose ultrafiltration of a solution comprising an organic polymer such as a Pluronic® block copolymer. Yet it was known that an organic polymer causes precipitation and limits concentration with ultrafiltration! *See* specification, page 1, lines 14-20. Therefore, evidence of high concentration without an organic polymer does not at all suggest or teach high concentration with an organic polymer present.

Furthermore, claim 1 has been clarified to recite that the second retentate is prepared ***without the removal of the organic polymer***. Thus, the claims clearly limit the process to one in

which ultrafiltration occurs on a solution that contains an organic polymer. Yet the art relied upon by the Patent Office does not show ultrafiltration of such a solution. Accordingly, reconsideration of the rejection is requested.

None of the patents cited at the sections referred to for showing high concentration starts with an organic polymer in the solution. Specifically, the Patent Office relies on example 4 of US 4,900,673 (Harper et al.), which discloses purification of angiogenin 100-fold by membrane ultrafiltration. However, this example does not disclose ultrafiltration of a solution containing an organic polymer such as a Pluronic® copolymer. In example 4, the cells were collected by centrifugation, treated, sonicated, and the resulting insoluble material treated in several steps including precipitation and washing before subjected to ultrafiltration. None of the treatment steps involved an organic polymer such as a Pluronic® block copolymer. Furthermore, such an organic polymer is not found in the disclosure of the cell culture. The cell line was grown in M9 media with certain supplements. Col. 10, lines 16-27. M9 media is composed of various salts, acids and glucose. According to public information on the composition of M9 media, it does not contain an organic polymer as recited in the present claims. Accordingly, although example 4 may disclose 100 fold concentration of protein by ultrafiltration, it does not disclose the presence of an organic polymer in the solution and, therefore, does not render obvious the high concentration obtained by Applicants' claimed process in which the organic polymer is present.

Furthermore, the other two references cited by the Patent Office in support of the obviousness of 50-fold or 100-fold concentration increase also do not show the presence of an organic polymer during ultrafiltration at the sections cited. The Office cites US 5,108,920 (Ng et al.) at Figure 7, which is an SDS-PAGE analysis of radioiodated retrovirus particles. Col. 6, lines 44-62. Ng et al. discloses that these retrovirus particles were purified "by equilibrium sucrose density gradient centrifugation." Col. 6, lines 45-46. Thus, Figure 7 does not show either high concentration by ultrafiltration, or a solution with an organic polymer. Accordingly, Applicants are confused by the Office's reference to Figure 7 as it seems irrelevant.

The Patent Office also cites US 5,567,602 (Clark et al. – erroneously cited in Office Action as US 51567602) at example 7.1. Office Action, page 12. Yet example 7.1 also fails to

disclose ultrafiltration of a solution containing an organic polymer such as that claimed. Example 7.1 shows extensive purification of a fusion protein, including amylase affinity chromatography, buffer exchange, and 100-fold dilution in an oxidizing buffer ***before*** 100-fold concentration by ultracentrifugation. Col. 25, line 56 to col. 26, line 6. Applicants respectfully do not see any mention of the presence of an organic polymer in the solution subjected to ultrafiltration. The person of ordinary skill in the art would review this disclosure of extensive purification ***before*** ultrafiltration and be led to assume that high concentration by ultrafiltration requires extensive pre-purification, or must follow a similar high level of dilution with a buffer, and could not be obtained if contaminants such as organic polymers are present. Thus, not only does Clark et al. not teach ultrafiltration of a solution containing an organic polymer, it actually teaches away from ultrafiltration of solutions containing contaminants such as organic polymers and away from the presently claimed process if high concentration is to be obtained because of the teachings of extensive prepurification and dilution with buffer before ultrafiltration.

In sum, the cited art does not teach or suggest that when using a macromolecule solution containing an organic polymer, ultrafiltration can provide a concentration of at least 50 fold or at least 100 fold.

Response to the Examiner's Rebuttal of Applicants' Prior Remarks

The Patent Office finds unpersuasive Applicants' prior discussion of Moller et al. and the multiple and unlikely modifications required to the Moller et al. process to obtain Applicants' claimed process. The Patent Office rejects Applicants' summary of Moller et al. as teaching primarily purification of only one protein. The Office Action states that the examiner is unaware of a legal standard that renders an obviousness teaching obsolete because it relies on a single example. Office Action, p. 6-7.

Yet Applicants' summary of Moller et al. was part of the *Graham v. John Deere Co.* inquiry of ascertaining the differences between the claimed invention and the prior art. Each prior art reference must be evaluated ***as an entirety***. *In re Evanega,* 829 F.2d 1110, 4 USPQ2d 1249 (Fed. Cir. 1987) (emphasis added). "Ascertaining the differences between the prior art and

the claims at issue requires interpreting the claim language, and ***considering both the invention and the prior art references as a whole***." MPEP 2141.02 (emphasis added). The test for obviousness is what the combined teachings of the references ***would have suggested to one of ordinary skill in the art***. *In re Young*, 927 F.2d 588, 18 USPQ2d 1089 (Fed. Cir. 1991) (emphasis added). Moller et al. cannot be dissected into bit pieces to support an obviousness rejection, but must be read as a whole with the view to understanding what it would have suggested to one of ordinary skill in the art. As an entirety, Moeller et al. teaches purification of hirudin expressed in yeast.

The Patent Office, however, disputes this summary of Moller et al. The Patent Office asserts instead that Moller et al. teaches that recombinant hirudin is only one example of the proteins that can be purified by the disclosed ultrafiltration process, and that the reference teaches generalizing this example to "any other recombinant expression system wherein production of proteins or peptides is desired." Office Action, p. 8.

Yet the evidence the Patent Office has pointed to as suggesting generalizing Moller et al. to other proteins and other expression systems offers little support for the Office's position. The cited teaching is found under "Background of the Invention," subheading "Description of the Related Art" and not under the "Detailed Description of the Invention." *See* Office Action, pp. 7-8, citing col. 1, lines 19-35 of Moller et al. The Patent Office cites to the third paragraph of the specification, which states that recombinant proteins require isolation from fermentation medium and "[o]ften" the protein has an overall charge. The patent then states that a "preferred" protein has an overall charge of 2 or larger. The patent next states, and the Patent Office quotes, that the utility of its process "may be demonstrated by the isolation of the thrombin inhibitor hirudin." Office Action at p. 8, citing Moller et al., col 1, lines 19-35. This passage from Moller et al. allegedly clearly states that hirudin in yeast is "an exemplary example." Office Action, p. 8. The Patent Office concludes from this passage that Moller et al. "clearly" states that hirudin expressed in yeast "is just an example which can be utilized in any other recombinant expression system wherein production of proteins or peptides is desired." Office Action, p. 8.

Yet this language is hardly a clear suggestion to generalize from hirudin. It allows that there may be other proteins that work by mentioning charged proteins as preferred, but does not provide an explanation of what proteins in what systems would be expected to work. Rather than reading the reference as a whole as the legal standard of obviousness requires, the Patent Office has dissected the reference and selected a general introduction and interpreted it as an assertion that the process should be generalized to any other expression system and any other protein.[1]

As Applicants previously showed, Moller et al. does not explain why its process defies conventional understanding to allow ultrafiltration with a membrane having much larger pores than the molecule to be retained. Without a mechanistic explanation, the person of ordinary skill in the art would not be likely to generalize the process beyond the protein and expression system disclosed and to have an expectation of success. Thus, the skilled artisan would not be motivated to combine Moller et al. with the other references relied upon to arrive at Applicants' claims.

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[1] When read in full context, the cited teaching for using the hirudin example in any other expression system for any other protein is really just an introduction to the general subject matter of the reference, as can be seen from the following longer excerpt incorporating the relied-upon text (col. 1, lines 13-47):
"Description of the Related Art

To isolate and purify a protein or peptide from fermentation medium, for example, by means of chromatographic methods, a prepurification must first be carried out. In many cases, this comprises desalination of the fermentation medium.

The need to isolate a protein or peptide from fermentation medium typically arises in the context of recombinant microorganisms transformed with suitable expression vectors. Desired proteins or peptides, for the present ultrafiltration process, are typically a recombinantly produced protein or peptide. Often, the desired protein or peptide may contain an overall charge due to a greater overall concentration of basic or acid amino acid residues. A preferred protein or peptide contain an overall positive or negative charge of 2 or larger. Also preferred is a protein or peptide with an overall charge of 4 or larger.

The utility of this process may be demonstrated by the isolation of the thrombin inhibitor hirudin, a single-chain protein with 65 amino acids, from the culture supernatant of the yeast strain Saccharomyces cerevisiae modified by genetic engineering.

The polypeptide hirudin, originally isolated from the leech Hirudo medicinalis, is a highly specific thrombin inhibitor with a broad therapeutic potential (F. Markward, Biomed. Biochim. Acta 44 (1985) 1007-1013). Hirudin is characterized by a high proportion of dicarboxylic acids. Isolation from native sources is not commercially practical in light of the amounts required for medical utility; such an amount can be prepared only by a genetic engineering route via transformed microorganisms. It has been found in this context that the yeast Saccharomyces cerevisiae is a suitable host organism for producing correctly folded and fully active hirudin (EP A1 168 342, EP A1 200 655)."

The Patent Office disagrees and cites passages from Moller et al. that, although possibly allowing for the skilled person to hypothesize on the reason for success, do not commit the invention to a mechanism of action. Specifically, the Patent Office cites again to the "Background of the Invention," under the subheading "Description of Related Art," rather than to the "Detailed Description of the Invention," to a disclosure that often the protein needing to be purified "may contain an overall charge" (Office Action, p. 9, citing to col. 1, lines 24-30):

> Often, the desired protein or peptide may contain an overall charge due to greater overall concentration of basic or acid amino acid residues. A preferred protein or peptide contain [sic] an overall positive or negative charge of 2 or larger. Also preferred is a protein or peptide with an overall charge of 4 or larger.

The Patent Office concludes from this that Moller et al. teaches "a sufficient explanation ***which can be transcended to most any protein*** and Applicants have merely overlooked said explanation." Office Action, p. 9, emphasis added. Yet this mere mention of preferred proteins having a charge is not an explanation of why the method works. There is no disclosure expressly stating that charge is the reason the large pore size membranes work. In fact, Moller et al. characterizes ultrafiltration membranes by their nominal molecular weight cut-off and does not mention charge of the membrane.

Furthermore, an ordinary skilled scientist looks for experiments to establish or at least suggest a mechanism. Moller et al. provides no such experiments. There is no report of scientific experiments to establish why the process works, let alone to establish for the scientific reader that the process works because of charge. A skilled person could hypothesize from this mention in the background section of a preference for charged proteins that charge is possibly the reason the process works. But Applicants respectfully submit this passage is not a disclosure of the mechanism of the process, let alone a "sufficient explanation" to generalize the process to "most any protein." Office Action, p. 9. ***When read as a whole***, Moller et al. does not encourage modification to other expression systems and proteins because the skilled reader does not find any scientific reasoning or evidentiary proof of the mechanism by which its process defies convention, only an inference of a hypothesis that charge on the protein is preferred.

Applicants provided many reasons in their prior response for the lack of motivation in the art to modify Moller et al. These explanations analyzed the Office's reasons for concluding motivation, and showed that the references do not support it. Yet in the Office Action, such remarks were characterized incorrectly as distinctions relying on unclaimed elements of the claims.

For example, the Office Action states that the Moller et al. system ***and the instant methods*** "are not limited by the expression system whatsoever." Office Action, p. 8. Whether the claimed methods are limited to a particular expression system is not relevant to motivation. Relevant is whether the skilled person would be motivated to change the expression system in Moller et al. Because the Patent Office's conclusion of obviousness requires motivation to change Moller et al. to an insect or mammalian system, which then would bring in references teaching usefulness of an organic polymer, the choice of expression system in Moller et al. is relevant to obviousness, even if it is not in the present claims.

Another example of this occurs in the Patent Office discarding comments on modifying the expression system as only relevant to dependent claim 9. See Office Action at p. 10 and 11. Applicants respectfully disagree. A motivation to change the yeast expression system in Moller et al. to a mammalian or insect cell expression system is required if Schulz et al. and Palomares et al. are to be combined with Moller et al. Moller et al. does not teach using an organic polymer such as a Pluronic® block copolymer. The Office's rejection relies on modifying Moller et al. to cultivate animal or insect cells, where the secondary references teach that an organic polymer is useful. *See* Office Action, p. 4. Accordingly, for this rejection to stand, the Patent Office must show a motivation to go from yeast cells to mammalian or insect cells. Therefore, Applicants' explanation of why the ordinary skilled person would not be motivated to change expression systems is relevant to rejection of ***all*** the claims, not just claim 9.

The Patent Office similarly found unconvincing Applicants' prior explanation of the unlikely series of steps required to modify Moller et al. The Patent Office again stated that because the explanation referred to steps that were not in the present claims, like desalinizing, Applicants were arguing method steps that are not present in the claims. *See* Office Action, pp.

11-12. Yet again, the obviousness rejection relies upon a motivation to modify Moller et al. Once major aspects of the Moller et al. system are modified as the rejection requires, such as the expression system and the desired protein, it is reasonable to question whether the skilled person would then be motivated to alter or omit purification steps, such as desalinization.

It would not have been obvious to use an organic polymer such as Pluronic® copolymer in the cell culture medium in Moller et al. The Office's asserted motivation relies on gross generalization of Moller et al. to any other expression systems and any other protein. The reference does not suggest this to the skilled reader. Furthermore, the Office presumes that once these major changes are made to the Moller et al system, the skilled person would still be motivated to use the purification process described in the example and would have a significant expectation of success. The references when read as a whole do not support these conclusions. Accordingly, withdrawal of the obviousness rejection is urged.

CONCLUSION

In sum, the high concentration recited in the claims renders the invention nonobvious over the cited art. Also, the Patent Office applied an incorrect legal standard in piecemeal selection of teachings from Moller et al. and not reviewing it as a whole for what it would suggest to one skilled in the art. Lastly, the Patent Office has improperly disregarded Applicants' earlier statements concerning the problems with the asserted motivation to modify Moller et al. The Patent Office has improperly dismissed them as relevant only to claims having limitations corresponding to the technical features of the asserted motivation to modify Moller et al. The references when properly considered do not motivate the skilled artisan to modify Moller et al. and obtain the claimed process. Accordingly, reconsideration and withdrawal of the rejections and allowance of the claims is in order.

Filed herewith is an authorization for payment of an extension of time for three months.

No further fee is believed due. However, if a fee is due, please charge our Deposit Account No. 03-2775, under Order No. 07430-00191 from which the undersigned is authorized to draw.

Dated:

Respectfully submitted,

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